

Haemato-Biochemical Changes in *Staphylococcal* Species Induced Mastitis in Mouse Model⁺

P Krishnamoorthy^{1, 2*}, M L Satyanarayana¹, BR Shome², Suguna Rao¹, H Rahman²

¹Department of Veterinary Pathology, Veterinary College, Karnataka Veterinary Animal and Fisheries Sciences University (KVAFSU), Hebbal, Bengaluru, Karnataka, India

²ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Ramagondanahalli, Yelahanka, Bengaluru, Karnataka, India

Abstract

In this study, haemato-biochemical changes in mouse mastitis by inoculating *Staphylococcus epidermidis*, *S. chromogenes*, *S. haemolyticus* and *S. aureus* isolated from bovine milk was studied. Blood was collected by intracardiac route at 6, 12, 24, 48, 72 and 96 h. Haematological changes noticed were: white blood cells (WBC) decreased in *S. haemolyticus* and *S. chromogenes* at 48 h; *S. haemolyticus* and *S. aureus* at 96 h. *S. haemolyticus* showed increased lymphocyte at 24 and 48 h and in *S. aureus* at 48 h. Neutrophil was decreased in *S. haemolyticus* and *S. aureus* group. Glucose increased at 6 h with three Coagulase Negative *Staphylococcus* (CNS) species and *S. aureus*. Glucose and total protein increased in *S. chromogenes* infected mice. Globulin levels showed decrease in *S. epidermidis* and *S. aureus*. Albumin globulin ratio showed decreasing trend in *S. chromogenes* and *S. haemolyticus* inoculated mice. Aspartate aminotransferase (AST) level increased at 6 and 12 h in all infected groups. Alanine aminotransferase increased at 12 and 48 h in *S. chromogenes* and *S. aureus* respectively. Lactate dehydrogenase (LDH) increased at 6 h in all the infected mice. Thus, three CNS species induced haemato-biochemical changes in haemoglobin, WBC, neutrophil, glucose, total protein, AST and LDH which may be used as indicators for diagnosis of CNS species mastitis in mice.

Keywords: *Staphylococcus* species, mouse mastitis, haemato-biochemical changes

*Author for Correspondence E-mail: krishvet@gmail.com

INTRODUCTION

Bovine mastitis is one of the major disease conditions affecting dairy cattle worldwide. Subclinical mastitis is difficult to detect due to the absence of any visible indications and it has major cost implications. Early detection of subclinical mastitis will help in reducing the economic loss to dairy farmers. Coagulase negative staphylococcus (CNS) species have become more important agents for bovine mastitis in recent years. More than ten CNS species have been isolated from mastitis milk and commonly reported are *Staphylococcus chromogenes*, *S. simulans*, *S. haemolyticus*, *S. hyicus* and *S. epidermidis* [1]. The study of mastitis in bovines is costly and involves various ethical and social issues, especially, in India and also keeping the bovines in controlled environment is difficult. Hence, the bovine mastitis is mostly studied in laboratory

animal models like mice, rat and rabbits. Various studies have been carried out using the animal models with major mastitis pathogens like *Staphylococcus aureus*, *Streptococcus* species and coliforms but not with CNS. Keeping this in view, the present study was undertaken to know haematological and biochemical changes caused by CNS species and *S. aureus* in mouse mastitis which may help in early diagnosis of subclinical mastitis.

MATERIALS AND METHODS

Animals and Mastitis Induction

CNS species namely *Staphylococcus epidermidis*, *S. chromogenes* and *S. haemolyticus* and coagulase positive *S. aureus* were isolated from apparently healthy bovine milk. Timed pregnant (Day 12 to 15), one hundred and sixty eight Swiss albino mice

were procured from National Centre for Laboratory Animal Science, Hyderabad and were grouped into seven groups of six mice each containing forty two mice per organism. Animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Veterinary College, Bangalore and carried out as per committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines, Ministry of environment and forests, Government of India, New Delhi, India. The 2×10^4 cfu organisms per teat were inoculated in mice as described earlier [2]. Pups were allowed to suckle after 1 h of inoculation to simulate the natural conditions.

Haematology and Biochemical Analysis

At 6, 12, 24, 48, 72 and 96 h, mice were sacrificed by using overdose of anesthesia; blood was collected by intracardiac route in dipotassium ethylene diamine tetra acetate (EDTA) vials for haematology; serum vials for biochemistry and smear prepared in grease free glass slide. Estimation of haemoglobin, packed cell volume (PCV), red blood cell

(RBC) and white blood cell (WBC) counts by using Auto haematology analyzer BC-2800 Vet (Mindray, India) as per Wiess and Wardrop [3]. The differential leukocyte count by counting hundred cells in smear and expressed as percentage. Estimation of glucose by Glucose oxidase-per oxidase [4], total protein by modified Biuret [5], albumin by Bromocresol Green [6], globulin by total protein minus albumin, aspartate aminotransferase (AST) by Modified UV International Federation of Clinical Chemistry (IFCC), alanine aminotransferase (ALT) by Modified UV IFCC [7] and lactate dehydrogenase (LDH) by optimized Deutsche Gesellschaft fur Klinische Chemie [8] methods by using kits (M/s. Span Diagnostics Limited, Surat, India) and Semiauto analyzer, Biosystems (BTS 320).

Statistical Analysis

The data obtained from different groups were analyzed by one way analysis of variance [9] and by using statistical analysis system (SAS) software version 9.3, Mumbai, India [10].

Table 1: The Mean Haemoglobin and Packed Cell Volume in Mice after Intramammary Inoculation with Three Coagulase Negative Staphylococcus Species and *S. aureus*.

Time Point	Haemoglobin (g/dL)					Packed Cell Volume (%)				
	PBS	<i>S. epidermidis</i>	<i>S. chromogenes</i>	<i>S. haemolyticus</i>	<i>S. aureus</i>	PBS	<i>S. epidermidis</i>	<i>S. chromogenes</i>	<i>S. haemolyticus</i>	<i>S. aureus</i>
6 h	12.70±0.42 ^a	12.53±0.56 ^a	12.75±0.45 ^a	13.73±0.44 ^a	10.93±0.32 ^b	33.40±5.16	38.40±1.04	36.40±3.80	44.07±2.22	39.17±7.83
12 h	12.15±0.35	11.83±0.72	11.40±1.00	12.23±0.18	11.80±1.01	41.65±2.05 ^{ab}	36.40±2.91 ^b	37.65±4.55 ^{ab}	54.00±2.35 ^a	43.33±7.53 ^{ab}
24 h	11.57±0.44 ^b	14.40±0.25 ^a	11.50±0.58 ^b	12.20±0.21 ^b	11.60±0.29 ^b	42.13±1.60 ^{ab}	39.97±1.22 ^b	44.00±2.23 ^{ab}	47.93±0.27 ^a	49.27±1.76 ^a
48 h	11.95±0.35	12.40±0.80	12.17±1.06	12.30±0.21	11.87±0.22	40.15±1.55 ^{ab}	46.85±0.15 ^a	36.17±4.39 ^b	49.50±1.01 ^a	47.40±1.82 ^a
72 h	11.95±0.74	12.43±0.09	13.20±0.60	12.10±0.42	12.83±0.55	45.17±3.12	43.90±1.88	41.70±1.70	47.17±1.96	46.07±4.39
96 h	13.65±0.95	12.05±1.65	11.60±0.69	11.47±0.22	11.70±0.21	36.40±2.50 ^b	33.45±0.35 ^b	41.97±4.53 ^{ab}	50.43±0.77 ^a	50.53±1.59 ^a
Me-an	12.28±0.26	12.66±0.32	12.03±0.31	12.34±0.19	11.79±0.22	39.83±1.72	39.79±1.22	39.86±1.53	48.85±0.93	45.96±1.93
CI	11.77–12.79	12.03–13.29	11.42–12.64	11.97–12.71	11.36–12.22	36.46–43.20	37.40–42.18	36.86–42.86	47.03–50.67	42.18–49.74

Table 2: The Mean Red Blood Cell and White Blood Cell Counts in Mice after Intramammary Inoculation with Three Coagulase Negative Staphylococcus Species and *S. aureus*.

Time Point	Red Blood Cell Count (x10 ⁶ per µl)					White Blood Cell Count (x10 ³ per µl)				
	PBS	<i>S. epidermidis</i>	<i>S. chromogenes</i>	<i>S. haemolyticus</i>	<i>S. aureus</i>	PBS	<i>S. epidermidis</i>	<i>S. chromogenes</i>	<i>S. haemolyticus</i>	<i>S. aureus</i>
6 h	7.62±0.7 9	7.84±0.17	7.70±0.30	8.14±0.44	7.44±1.43	8.87±0.83	8.81±0.23	8.44±0.01	7.13±0.32	8.65±1.85
12 h	8.50±0.18	7.37±0.49	7.59±0.75	10.13±0.36	8.25±1.33	8.39±0.43	9.13±0.56	8.87±0.85	6.03±0.29	7.86±1.49
24 h	8.71±0.07	8.01±0.21	8.29±0.39	8.67±0.04	8.89±0.38	7.85±0.52 ^{ab}	8.53±0.33 ^a	7.68±0.24 ^{abc}	6.53±0.04 ^{bc}	6.28±0.23 ^c
48 h	8.45±0.32 ^{ab}	9.45±0.19 ^a	7.03±0.75 ^b	9.79±0.73 ^a	8.47±0.25 ^{ab}	8.56±0.50 ^{ab}	7.37±0.23 ^{bc}	9.74±0.94 ^a	6.18±0.52 ^c	6.55±0.27 ^{bc}
72 h	8.87±0.38	7.65±0.74	8.15±0.01	8.93±0.59	8.21±1.01	7.68±0.74	7.94±0.60	7.43±0.99	6.79±0.16	7.22±0.89
96 h	7.37±0.45 ^c	6.98±0.65 ^c	7.90±0.56 ^{bc}	9.60±0.24 ^a	9.02±0.25 ^{ab}	9.18±0.46 ^a	8.20±0.71 ^{ab}	7.28±1.22 ^{ab}	6.38±0.17 ^b	6.22±0.27 ^b
Me-an	8.28±0.24	7.84±0.24	7.77±0.22	9.21±0.23	8.38±0.34	8.35±0.29	8.39±0.21	8.08±0.34	6.51±0.13	7.13±0.42
CI	7.81–8.75	7.37–8.31	7.34–8.20	8.76–9.66	7.71–9.05	7.78–8.92	7.98–8.80	7.41–8.75	6.26–6.76	6.31–7.95

Table 3: The Mean Lymphocyte and Neutrophil Counts in Mice after Intramammary Inoculation with Three Coagulase Negative Staphylococcus Species and *S. aureus*.

Time Point	Lymphocyte (%)					Neutrophil (%)				
	PBS	<i>S. epidermidis</i>	<i>S. chromogenes</i>	<i>S. haemolyticus</i>	<i>S. aureus</i>	PBS	<i>S. epidermidis</i>	<i>S. chromogenes</i>	<i>S. haemolyticus</i>	<i>S. aureus</i>
6 h	78.65±1.42	78.57±0.46	79.55±0.15	80.83±0.29	79.13±2.22	18.92±1.26	18.93±0.40	18.15±0.05	17.07±0.23	18.53±1.93
12 h	79.60±0.60	77.80±0.30	77.70±0.50	81.47±0.07	78.97±1.94	18.10±0.50	19.70±0.30	19.80±0.50	16.50±0.10	18.73±1.69
24 h	79.30±0.56 ^{ab}	78.43±1.07 ^b	79.80±0.25 ^{ab}	81.23±0.14 ^a	80.67±0.26 ^{ab}	18.37±0.48	19.13±0.99	17.93±0.22	16.73±0.12	17.23±0.30
48 h	78.40±0.80 ^c	79.80±0.1 ^{abc}	78.97±0.99 ^{bc}	81.43±0.48 ^a	80.83±0.14 ^{ab}	19.15±0.75 ^a	17.90±0.10 ^{abc}	18.63±0.84 ^{ab}	16.53±0.42 ^c	17.03±0.12 ^{bc}
72 h	81.82±1.47	80.27±0.72	79.95±1.25	80.97±0.23	80.87±0.74	15.82±1.59	17.63±0.69	17.80±1.10	16.93±0.20	17.00±0.60
96 h	78.65±0.65 ^{ab}	77.40±1.00 ^b	80.60±1.20 ^a	81.33±0.38 ^a	81.33±0.09 ^a	18.90±0.60 ^{ab}	19.75±0.45 ^a	17.23±1.03 ^{bc}	16.77±0.33 ^{bc}	16.60±0.06 ^c
Me-an	79.40±0.52	78.71±0.46	79.43±0.41	81.21±0.10	80.30±0.40	18.21±0.50	18.84±0.29	18.19±0.33	16.75±0.10	17.52±0.42
CI	78.38–80.42	77.81–79.61	78.63–80.23	81.01–81.41	79.52–81.08	17.23–19.19	18.27–19.41	17.54–18.84	16.55–16.95	16.70–18.34

Values were expressed as Mean ± Standard error.

CI: Confidence interval; ^{a, b, c}: Means with same superscript within the row do not differ significantly ($P>0.05$).

Table 4: The Mean Serum Glucose and Total Protein Levels in Mice after Intramammary Inoculation with Three Coagulase Negative Staphylococcus Species and *S. aureus*.

Time Point	Glucose (mg per dL)					Total Protein (g per dL)				
	PBS	<i>S. epidermidis</i>	<i>S. chromogenes</i>	<i>S. haemolyticus</i>	<i>S. aureus</i>	PBS	<i>S. epidermidis</i>	<i>S. chromogenes</i>	<i>S. haemolyticus</i>	<i>S. aureus</i>
6 h	70.97±11.24 ^c	114.43±10.01 ^{ab}	152.54±8.04 ^a	102.46±11.25 ^{bc}	129.43±12.09 ^{ab}	4.26±0.09 ^b	4.09±0.04 ^b	6.76±0.19 ^a	6.75±0.11 ^a	4.47±0.12 ^b
12 h	87.66±10.66	83.34±6.45	110.70±8.77	86.09±12.71	103.87±13.44	4.63±0.26 ^c	4.03±0.19 ^d	7.24±0.13 ^a	6.29±0.05 ^b	4.20±0.09 ^{cd}
24 h	75.22±8.27	77.91±9.50	130.43±7.89	109.72±4.26	127.69±13.92	4.36±0.55 ^c	4.68±0.20 ^c	7.29±0.26 ^a	6.32±0.23 ^{ab}	5.14±0.48 ^{bc}
48 h	88.75±10.51 ^b	90.51±8.09 ^b	134.37±9.00 ^a	97.04±8.30 ^{ab}	109.60±19.82 ^{ab}	5.46±0.35 ^{bc}	4.92±0.41 ^{bc}	7.76±0.43 ^a	5.94±0.45 ^b	4.33±0.14 ^c
72 h	90.30±7.08 ^{bc}	90.67±4.65 ^{bc}	139.63±14.87 ^a	114.11±8.62 ^{ab}	67.34±8.96 ^c	4.65±0.17 ^{bc}	4.06±0.27 ^c	7.17±0.25 ^a	5.36±0.15 ^b	5.19±0.39 ^b
96 h	96.94±15.59 ^b	105.96±2.20 ^{ab}	135.41±14.44 ^a	108.96±1.97 ^{ab}	82.42±9.51 ^b	4.75±0.21 ^c	4.60±0.08 ^{cd}	7.04±0.22 ^a	5.51±0.07 ^b	3.98±0.39 ^d
Me-an	83.58±4.16	92.76±3.93	134.53±5.13	103.35±3.30	103.39±7.84	4.64±0.13	4.46±0.12	7.26±0.14	6.00±0.14	4.55±0.14
CI	75.43–91.73	85.06–100.46	124.48–144.58	96.88–109.82	88.02–118.76	4.39–4.89	4.23–4.69	6.99–7.53	5.73–6.27	4.28–4.82

Values were expressed as Mean ± Standard error.

^{a, b, c, d}: Means with same superscript within the row do not differ significantly ($P > 0.05$);

CI: Confidence interval at 95 per cent level.

Table 5: The Mean Serum Albumin, Globulin and Albumin Globulin Ratio in Mice.

Time Point	Albumin (g per dL)					Globulin (g per dL)					Albumin Globulin Ratio				
	PBS	<i>S. epidermidis</i>	<i>S. chromogenes</i>	<i>S. haemolyticus</i>	<i>S. aureus</i>	PBS	<i>S. epidermidis</i>	<i>S. chromogenes</i>	<i>S. haemolyticus</i>	<i>S. aureus</i>	PBS	<i>S. epidermidis</i>	<i>S. chromogenes</i>	<i>S. haemolyticus</i>	<i>S. aureus</i>
6 h	2.34±0.13 ^b	2.55±0.07 ^{ab}	2.98±0.29 ^a	2.41±0.17 ^b	2.56±0.09 ^{ab}	1.92±0.17 ^c	1.54±0.05 ^c	3.79±0.09 ^b	4.33±0.08 ^a	1.91±0.14 ^c	1.30±0.09 ^{ab}	1.67±0.10 ^a	0.79±0.09 ^{bc}	0.56±0.05 ^c	1.38±0.14 ^a
12 h	2.38±0.15	2.65±0.30	2.81±0.11	2.87±0.11	2.32±0.13	2.25±0.33 ^c	1.38±0.42	4.43±0.13	3.42±0.15	1.88±0.11	1.14±0.09 ^{ab}	2.67±1.26 ^a	0.63±0.33 ^b	0.84±0.07 ^b	1.27±0.15 ^{ab}
24 h	2.31±0.37	2.64±0.15	2.83±0.33	2.56±0.12	2.72±0.16	2.05±0.24 ^b	2.04±0.15 ^b	4.46±0.23 ^a	3.77±0.22 ^a	2.42±0.33 ^b	1.14±0.08 ^a	1.33±0.14 ^a	0.64±0.03 ^b	0.69±0.07 ^b	1.17±0.09 ^a
48 h	2.73±0.25	2.65±0.14	2.83±0.21	2.04±0.37	2.48±0.18	2.73±0.44 ^c	2.27±0.28 ^c	4.93±0.30 ^a	3.90±0.38 ^b	1.85±0.24 ^c	1.16±0.34 ^{ab}	1.20±0.09 ^a	0.58±0.05 ^b	0.57±0.13 ^b	1.47±0.26 ^a
72 h	2.67±0.16	2.34±0.15	2.55±0.33	2.44±0.18	2.64±0.11	1.98±0.25 ^{cd}	1.72±0.14 ^d	4.61±0.14 ^a	2.92±0.24 ^b	2.55±0.22 ^{bc}	1.50±0.24 ^a	1.37±0.08 ^{ab}	0.56±0.08 ^c	0.85±0.13 ^{bc}	1.05±0.3 ^{abc}
96 h	2.96±0.22	2.57±0.21	2.71±0.11	2.76±0.27	2.46±0.06	1.79±0.43 ^{bc}	2.03±0.22 ^{bc}	4.33±0.22 ^a	2.74±0.30 ^b	1.52±0.38 ^c	1.87±0.46 ^a	1.36±0.27 ^{ab}	0.63±0.05 ^b	1.14±0.11 ^{ab}	1.29±0.07 ^{ab}
Me-an	2.54±0.09	2.58±0.07	2.76±0.08	2.48±0.12	2.53±0.06	2.10±0.12	1.88±0.11	4.50±0.11	3.52±0.16	2.02±0.12	1.33±0.08	1.55±0.13	0.62±0.03	0.77±0.08	1.27±0.06
CI	2.36–2.72	2.44–2.72	2.60–2.92	2.25–2.71	2.41–2.65	1.87–2.33	1.67–2.09	4.29–4.71	3.21–3.83	1.15–1.39	1.13–1.53	1.20–1.90	0.56–0.68	0.61–0.93	1.15–1.39

Table 6: The Mean Serum Aspartate Aminotransferase, Alanine Aminotransferase and Lactate Dehydrogenase in Mice.

Time Point	AST (IU per litre)					ALT (IU per litre)					Lactate Dehydrogenase (IU per litre)				
	PBS	<i>S. epidermidis</i>	<i>S. chromogenes</i>	<i>S. haemolyticus</i>	<i>S. aureus</i>	PBS	<i>S. epidermidis</i>	<i>S. chromogenes</i>	<i>S. haemolyticus</i>	<i>S. aureus</i>	PBS	<i>S. epidermidis</i>	<i>S. chromogenes</i>	<i>S. haemolyticus</i>	<i>S. aureus</i>
6 h	10.82±1.79 ^b	19.39±0.86 ^a	21.22±1.77 ^a	21.16±0.78 ^a	21.27±0.92 ^a	24.45±1.78	23.41±1.82	29.89±1.05	22.17±4.64	26.32±5.10	217.14±11.89 ^{ab}	160.30±23.14 ^b	252.10±12.02 ^a	240.45±46.27 ^a	251.94±12.73 ^a
12 h	10.62±2.75 ^b	22.10±0.20 ^a	19.45±1.33 ^a	23.19±1.00 ^a	20.37±2.45 ^a	25.85±1.39 ^a _b	25.34±2.81 ^a _b	34.23±4.17 ^a	21.88±5.01 ^b	18.39±1.79 _b	195.27±10.49	240.32±23.14	180.34±60.11	229.76±25.49	217.52±24.40
24 h	14.38±0.71	20.84±3.26	17.68±3.57	16.64±1.59	17.37±1.72	27.32±6.16	24.59±1.28	22.32±4.67	24.29±3.00	25.09±3.75	242.93±7.25 ^{ab}	214.58±34.33 ^a _b	162.85±38.69 ^b	274.84±45.93 ^a	155.94±24.42 ^b
48 h	21.08±0.76	26.56±4.87	21.82±1.11	18.31±1.55	18.38±3.28	18.85±2.88 ^a _b	22.02±1.40 ^a _b	15.09±3.25 _b	20.62±2.96 ^a _b	24.80±2.10 ^a	225.48±9.59 ^{ab}	294.63±40.92 ^a	211.47±22.96 ^a _b	191.00±29.00 ^b	217.48±26.76 ^a _b
72 h	18.37±1.23 ^{ab}	25.49±0.64 ^a	22.36±3.80 ^a _b	16.50±3.24 _b	20.85±2.64 ^a _b	23.40±1.13	22.03±5.97	21.02±2.96	26.26±2.96	23.03±1.85	244.51±15.09	229.84±54.81	178.33±15.13	176.21±37.76	243.11±23.60
96 h	19.15±3.47 ^{ab}	24.22±0.98 ^a	22.88±1.45 ^a	15.29±3.03 _b	15.87±0.84 ^b	22.48±0.47	28.62±3.29	21.40±2.99	21.22±2.78	21.01±1.96	216.18±35.95 ^a _b	322.60±52.42 ^a	197.86±19.48 ^b	198.38±33.05 ^b	221.16±27.34 ^a _b
Mean	15.40±1.01	23.10±1.19	21.35±0.97	18.08±0.95	19.02±0.89	23.93±1.26	24.45±1.12	21.99±1.78	22.49±1.30	23.11±1.23	225.57±6.33	246.97±18.72	195.88±10.90	218.05±15.50	217.86±10.49
CI	13.42–17.38	20.77–25.43	19.45–23.25	16.18–19.98	17.28–20.76	21.46–26.40	22.26–26.64	18.50–25.48	19.94–25.04	20.70–25.52	212.86–237.98	210.28–283.66	174.55–217.24	187.67–248.43	197.30–238.42

Values were expressed as Mean ± Standard error.

^{a, b, c}; Means with same superscript within the row do not differ significantly ($P>0.05$), CI—Confidence interval at 95 per cent level.

RESULTS

Haematological Changes

Haemoglobin, PCV; RBC, WBC and lymphocyte, neutrophil counts were presented in Tables 1–3 respectively. At 6 h, haemoglobin showed significant ($P<0.05$) decrease in *S. aureus* infected mice. At 12 h, PCV showed significant ($P<0.05$) decrease in *S. epidermidis* infected mice. PCV revealed significant ($P<0.05$) increase in *S. haemolyticus* and *S. aureus* infected mice at 24 and 48 h. RBC count showed significant ($P<0.05$) increase in *S. epidermidis* and *S. haemolyticus* inoculated mice at 48 h and *S. haemolyticus* and *S. aureus* at 96 h. WBC count showed significant ($P<0.05$) increase in *S. epidermidis* and *S. aureus* inoculated mice at 24 h. At 96 h, WBC count showed significant ($P<0.05$) decrease in *S. haemolyticus* and *S. aureus* infected mice.

Lymphocyte count increased in *S. haemolyticus* and decreased in *S. epidermidis* at 24 h. At 48 h, *S. aureus* and *S. haemolyticus* showed significant ($P<0.05$) increase in lymphocyte count. Neutrophil count showed significant ($P<0.05$) decrease in *S. haemolyticus* and *S. aureus* at 48 h and in *S. aureus* at 96 h.

Biochemical Changes

The glucose, protein; albumin, globulin, albumin globulin ratio and AST, ALT, LDH in mice are presented in Tables 4–6 respectively. At 6 h, glucose showed significant ($P<0.05$) increase in all the infected mice and in *S. chromogenes* at 48, 72 and 96 h. Total protein showed significant ($P<0.05$) increase in *S. chromogenes* infected mice at all time points. At 6 h, *S. chromogenes* showed significant ($P<0.05$) increase in albumin. Globulin

revealed significant ($P < 0.05$) decrease in *S. chromogenes* and *S. haemolyticus* at 6 h and increase in *S. chromogenes* at 24, 48, 72 and 96 h. Albumin globulin ratio showed decreasing trend in *S. chromogenes* and *S. haemolyticus*. Significant ($P < 0.05$) increase in AST at 6 and 12 h in all infected mice. ALT increased in *S. aureus* and decreased in *S. chromogenes* at 48 h. At 6 h, *S. chromogenes*, *S. haemolyticus* and *S. aureus* inoculated mice showed significant ($P < 0.05$) increase in LDH values.

DISCUSSION

Haematological Changes

No significant difference in haemoglobin levels at 12, 48, 72 and 96 h and corroborated with previous report [11]. *S. aureus* inoculated mice showed decrease at 6 h and *S. epidermidis* at 24 h in haemoglobin and concurred with previous study [12]. Zaki et al. concluded that sub clinical mastitis caused anaemia in dairy cows by decreasing the haemoglobin and RBC count [12].

S. haemolyticus and *S. aureus* showed increase in PCV at 24 and 72 h. RBC counts showed no difference at 6, 12, 24 and 72 h, which corroborated with previous report [11]. At 48 h, *S. haemolyticus* and *S. epidermidis* and at 96 h *S. haemolyticus* and *S. aureus* showed increase in RBC count. However, decrease in PCV and RBC counts in subclinical mastitis cases was observed in buffaloes [12].

This may be due to change in the microorganisms causing subclinical mastitis. WBC count showed increase in *S. epidermidis* and *S. chromogenes* at 24 and 48 h respectively and was concurred with previous reports [12, 13] and it might due to inflammation and stress.

The polymorpho neutrophils in the mammary gland act as first line of defense against invading mastitis pathogens [14]. At 96 h, *S. haemolyticus* and *S. aureus* infected mice showed significant decrease in WBC count and concurred with previously reported in *S. aureus* mastitis [11]. This might be due to decrease in the neutrophil numbers after initial increase upon intramammary infection and due to migration of neutrophils to mammary gland. Lymphocyte increased at 48 h in *S.*

haemolyticus and *S. aureus* inoculated mice. However; Holtenius et al. observed no changes in lymphocyte counts in mastitis herds [15] and Ohtsuka et al. reported decrease in peripheral lymphocytes in herds frequently affected with mastitis [13].

Biochemical Changes

S. chromogenes infected mice increased glucose and total protein but no difference in glucose was reported between high mastitis incidence and low mastitis incidence herds [15] and in *S. aureus* mastitis [11]. The increase in glucose concentration in early hours may be due to action of cortisol released during stress and microorganisms. CNS inoculated mice showed no difference in total protein levels and concurred with previous findings [11]. Albumin showed no significant difference in CNS and *S. aureus* which corroborated with previous report [11].

Globulin increased in *S. chromogenes* infected mice and it might be due to increase in total protein. AST revealed increase at earlier time points and concurred with previous studies [12, 16]. This may be attributed to mammary gland and liver damage in CNS mastitis. Moyes et al. indicated AST may serve as potential indicator for assessment of risk of mastitis during early lactation in dairy cattle [16]. No difference in ALT was observed and concurred with previous report [12]. LDH increased in earlier time points and corroborated with Wenz et al.; indicated 1.3 times LDH activity in moderate to severe mastitis in dairy cows [17]; and Zaki et al. reported significant elevation of LDH in infected milk [12].

CONCLUSION

This study, successfully established the CNS mastitis in mouse model. CNS species induced haematological and biochemical changes in mouse mastitis, as compared to *S. aureus*. Haemoglobin, WBC and neutrophil count, glucose, total protein, AST and LDH may be used as indicators for early diagnosis of CNS mastitis. Further research is needed to investigate the haematology and biochemical changes in CNS mastitis in dairy cows.

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